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INVESTIGATION OF THE ABSORPTION CHANGES OF THE PLASTOQUINONE SYSTEM IN BROKEN CHLOROPLASTS

THE EFFECT OF BICARBONATE-DEPLETION

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SUMMARY

The absorption changes at 334, 265, and 703 nm were measured in chloroplast suspensions upon depletion and readdition of bicarbonate. The normal absorption changes in the ultraviolet region are reinterpreted taking into account the existence of an additional plastoquinone molecule R located between the primary electron acceptor Q and the plastoquinone pool PQ. On this basis, three reversible effects of bicarbonate depletion were located in the electron transfer chain:

(1) The reaction centers II are partly inactivated as observed by the reduced magnitude of absorption changes of a quinone X-320 (or Q).

(2) The formation of R^{2-} is retarded by a factor of 10–15 (from 500** μs to 7 ± 3 ms), as observed by the dark relaxation of the absorption change at 334 nm. This is consistent with the slowing down of the dark relaxation of the oxygen evolving S states (Sn′ to Sn+1) from a value of approx. 500 μs to 10 ms (observed by Stemler et al. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4679–4683).

(3) The reduction of plastoquinone by R^{2-} is slowed down from approximately a ms to 100–200 ms*** in agreement with the interpretation of chlorophyll *a* fluorescence decay after the third flash (150 ms; Govindjee et al. (1976) Biochim. Biophys. Acta 449, 602–605) .This slow reaction time is apparent in both the absorption changes of *P*-700 (reaction center I) and plastoquinone after a 20 μs flash (with an alteration from a predominating approx. 50 ms phase to a predominating approx. 200 ms phase) and also in the plastoquinone signal after an 85 ms flash (from 25 to 100 ms). This deceleration is the major effect of bicarbonate depletion and must be associated with the 4 to 8-fold loss in Hill activity observed by earlier workers.

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** The reduction of R by Q^- (or the decay of Q^- to Q) occurs with a halftime ranging from 400 to 600 μs; in this paper we shall call this 500 μs for ease in reading the text.

*** The reduction of plastoquinone pool by R^{2-} in bicarbonate-depleted chloroplasts ranges from a halftime of 170–230 ms (in short flashes) and from 75 to 100 ms (in long flashes).

INTRODUCTION

Bicarbonate* depletion of chloroplasts greatly reduces the overall electron transport as measured by the Hill reaction [1–5]; at least one site of this inhibition is on the reducing side of Photosystem II [6]. Measurements of the decay of chlorophyll *a* fluorescence yield, after light flashes, suggest that the reoxidation of the reduced "primary" acceptor Q^- [7] and, to a larger extent, the reoxidation of the reduced secondary acceptor (R^{2-}) by the plastoquinone (PQ) pool [8] are inhibited by bicarbonate-depletion.

It seemed desirable to check the above conclusions by directly monitoring the absorption changes corresponding to individual electron transfer reactions: The absorption changes of the plastoquinone system (Q, R, and PQ) and of *P*-700 (chlorophyll-a_1) were measured. Whereas evaluation of changes at 703 nm for *P*-700 is straightforward, the same in the ultraviolet region for plastoquinone is not. The simplest possibility was to regard the latter changes to be due to the sum of the following reactions [9–12]: (1) the transition of quinone to semiquinone anion of the "primary" acceptor X-320 (Q) of light reaction II; and (2) the transition of quinone to hydroquinone of the PQ pool molecules. Recently, however, the involvement of a secondary acceptor R or B has been shown [13–15]. If this R is a special plastoquinone, distinct from that in the pool, but with absorption changes in the ultraviolet region [16], the measured ultraviolet absorption changes must be reinterpreted, as was begun in ref. 17. The superposition of three absorption changes leads to several kinetic phases in the observed signals. In this paper, absorption changes at 334 nm, not at 320 nm, were taken to indicate the formation and decay of semiquinone anion (Q^-), since we found nearly the same signals in bicarbonate-depleted and control chloroplasts at both these wavelengths. Changes in absorption at 265 nm indicated changes in R and the PQ pool. Data on control, bicarbonate-depleted and reconstituted chloroplasts are presented.

MATERIALS AND METHODS

Class II chloroplasts from spinach (*Spinacea oleracea*) were depleted of bicarbonate as described elsewhere [6]. The reaction mixture (at pH 6.8) contained 100 mM sodium formate, 100 mM NaCl, 50 mM sodium phosphate, 0.5 mM $K_3Fe(CN)_6$, 0.02 mM gramicidin, 0.1 mM chlorophyll, and 20 mM sodium bicarbonate, where indicated. The absorption changes were measured with a double beam difference spectrophotometer for simultaneous measurements at two wavelengths with repetitive excitation and signal averaging (Fabri-Tek 1072), as described in ref. 18. Long (85 ms) light flashes were provided by the combination of an actinic lamp and a shutter and short (20 μs) light flashes by a flash lamp. The monitoring ultraviolet light was from a xenon-mercury high pressure arc lamp (Hanovia). The cuvette was flat (thickness, 1 mm) and was traversed by the monitoring light at an angle of 45 °.

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* CO_2 and bicarbonate have been interchangeably used in the text without reference to the active species involved.

RESULTS AND DISCUSSION

1. *Absorption changes at 334 nm*

The signal at 334 nm shows a very fast rise (< 1 μs; [10, 19]) and a biphasic dark relaxation dominated by an exponential phase with a half-lifetime of ~ 500 μs (Fig. 1, top). The fast rise is attributed to the rapid photoreduction of Q and the 500 μs decay to the subsequent thermal reoxidation of Q^-. A minor portion of the signal (10–15 %), with a half-lifetime of 10–20 ms, is due to an uncharacterized signal, probably from the formation of plastohydroquinone or the reduction of oxidized *P*-700. The reoxidation of Q^- in the dark was originally regarded as being brought about by dismutation of a twin of two Q^- [10] and later by a double electron transfer from two Q^- to the PQ-pool [20]. By the introduction of an additional connector molecule R between Q and PQ [13–15], two processes must be summed up:

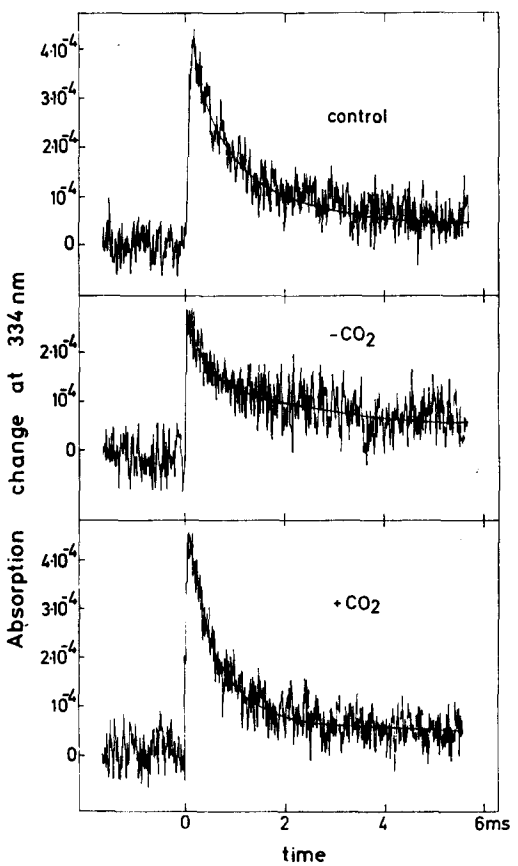


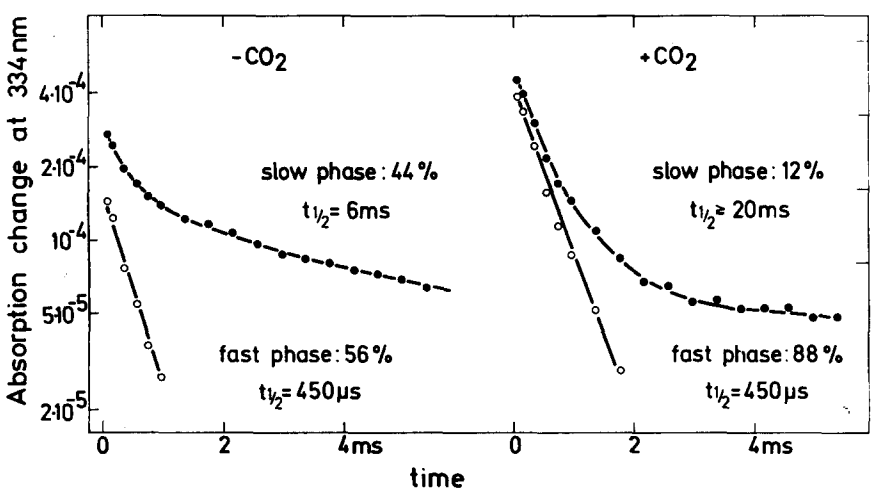
Fig. 1. Time course of the absorption change at 334 nm induced by 20 μs repetitive flashes (indicating mainly Q^- and R^-) for untreated chloroplasts (frozen and rethawed, control), after bicarbonate. depletion ($-CO_2$) and after readdition of bicarbonate to a final concentration of 20 mM ($+CO_2$). The amplitudes $\Delta I/I$ are $4.2 \cdot 10^{-4}$ $2.7 \cdot 10^{-4}$ and $4.5 \cdot 10^{-4}$, respectively. 512 flashes were averaged; darktime between the flashes (t_d), 250 ms. Electrical bandwidth, 10 kHz.

$$Q^- + R \xrightarrow{k_1} Q + R^- \quad (1)$$

$$Q^- + R^- \xrightarrow{k_2} Q + R^{2-} \quad (2)$$

Without any assumptions, biphasic decay kinetics are expected from the above reactions. The amplitudes of the phases corresponding to k_1 and k_2 should be proportional to $(\Delta\varepsilon_Q - \Delta\varepsilon_R)$ and $(\Delta\varepsilon_Q - [\Delta\varepsilon_R{}^{2-} - \Delta\varepsilon_R])$, where $\Delta\varepsilon_Q$, $\Delta\varepsilon_R$, and $\Delta\varepsilon_R{}^{2-}$ are the extinction coefficient differences for the redox systems Q^-/Q, R^-/R and R^{2-}/R, respectively. In the repetitive flash technique, with averaging of a large number of signals, we should observe both signals equally (50 %). The decay of the signal at 334 nm with one dominant fast phase in spite of two reactions may be explained in two extreme ways: (a) if $\Delta\varepsilon_Q$ and $\Delta\varepsilon_R$ are assumed to be markedly distinct, then k_1 and k_2 must be equal (as was assumed in ref. 15); or (b) if these $\Delta\varepsilon$-values are equal, then the observed decay kinetics reflect the reaction of only those systems which are in the state Q^-R^-, i.e., the process determined by k_2. In both cases, according to ref. 9, $\Delta\varepsilon_R{}^{2-}$ is taken to be negligible. It is assumed here that both Q^- and R^- are plastosemiquinone anions. (We do not take into account possible differences in protonation.) Equality or minor differences between $\Delta\varepsilon_Q$ and $\Delta\varepsilon_R$ are expected on this basis. This excludes explanation (a). In any case, more than 50 % of the signal must be determined by k_2; this decay represents the disappearance of two semiquinones and the formation of one hydroquinone.

Bicarbonate depletion (Figs. 1 and 2) had two effects on the absorption change at 334 nm: (1) the amplitude is reduced to 60–65 % of the control which is in agreement with the reduction of the absorption change of Chlorophyll-a_{II} (P-680) [7] and reflects inhibition of reaction centers II; and (2) the decay becomes clearly biphasic. It is described by two exponential phases of about equal magnitude with half-lifetimes ($t_{\frac{1}{2}}$) of: 500±100 μs, and 7±3 ms (average of 5 experiments). Second order kinetics can be excluded on the basis of a plot of $[\Delta A(t)]^{-1}$ vs. t (not shown). Both effects of



Figs. 2. Semilogarithmic plot of the $\pm CO_2$ curves of Fig. 1. The relative magnitude and halftime of the two exponential phases are indicated.

CO_2-depletion are reversible; the original signal is restored to a fair degree by the readdition of bicarbonate (Figs. 1 and 2).

If the reoxidation time of R^- in CO_2-depleted chloroplasts is the same as in control chloroplasts (about a few minutes; Velthuys, [21], then there are two possible interpretations of the modified kinetics. The fact that the original halftime is retained for half of the amplitude in the depleted samples could have implied that one of the two reactions mentioned above remains unchanged, whereas the other' (1 or 2) is slowed down by a large factor. This corresponds to an extreme version of explanation (a) which has been excluded (see above) for the control kinetics (but with $k_1 \neq k_2$). To account for the observed amplitude ratio in depleted-chloroplasts (0.44 : 0.56) the differential extinction coefficients of Q and R would have to differ very much: $\Delta\varepsilon_Q \cong 8\ \Delta\varepsilon_R$. This interpretation is not reconciled with the current belief that both Q and R are special plastoquinone molecules. In the second interpretation, which we favor, the biphasic relaxation reflects heterogeneity of the signal introduced by our treatment. We suggest that approx. 50 % of the sample was unaffected in its decay kinetics by the procedure of CO_2-depletion, whereas the other 50 % exhibits modified kinetics. This interpretation is independent of assumptions for the extinction coefficients. Accordingly, we cannot say which of the reaction constants, k_2 or both k_1 and k_2, are reduced by bicarbonate (CO_2)-depletion. However, a change in both reaction constants is suggested by the fact that the electron transfer is between a Q^- and an R^- species in both cases and by the possibility that the change may be due to a topological reorganization.

We note that Jursinic et al. [7] reported $t_{\frac{1}{2}}$ of 2.6 ms for the reoxidation of Q^- to Q in CO_2-depleted chloroplasts. This may not be incompatible with the above result of 7 ± 3 ms if we accept that fluorescence decay monitors Q^- to Q reaction and the absorption change monitors mainly the reoxidation of Q^- by R^-. We may not, therefore, be surprised that the decay time of X-320, measured in the repetitive flash technique, is somewhat longer than the decay time of the fluorescence yield measured after a single saturating flash. However, Stemler et al. [5] observed that relaxation of $S_{n'}$ to S_{n+1} (due mainly to reoxidation of Q^- to Q) is decreased from a halftime of approx. 500 μs to 10 ms upon bicarbonate depletion. Addition of 10 mM bicarbonate restored the 500 μs component. Our data are closer to those of Stemler et al. [5].

2. *Absorption change at 265 nm induced by 20 μs repetitive flashes*

The dark relaxation of the absorption change at 265 nm, measured at pH 7–8, is composed of a minor (20 % amplitude) fast phase ($t_{\frac{1}{2}} = 500\ \mu$s) and a major slow phase ($t_{\frac{1}{2}} = 20$ ms) for osmotically shocked chloroplasts [10]. Normal class II chloroplasts exhibit a minor medium phase of about 5 ms with 20 % of the total amplitude, if the 500 μs phase is not resolved [20]; the interpretation of this new 5-ms phase is not yet clear. If only Q and PQ were involved in the absorption changes at 265 nm, the 20-ms time can only represent the oxidation of plastohydroquinone [10, 20]. With an additional molecule R [13–15], which has an absorption similar to PQ [16], this time may also be attributed to the transfer of the electron pair from R^{2-} to PQ. With this hypothesis, the absorption change after a short flash is primarily due to the formation of R^{2-}, whereas the plastohydroquinone in the pool formed thereafter is only a short-lived intermediate. As the absorption change induced by a long flash indicates the reduction of a large portion of the pool [10, 22], we know that reduction of PQ pool is

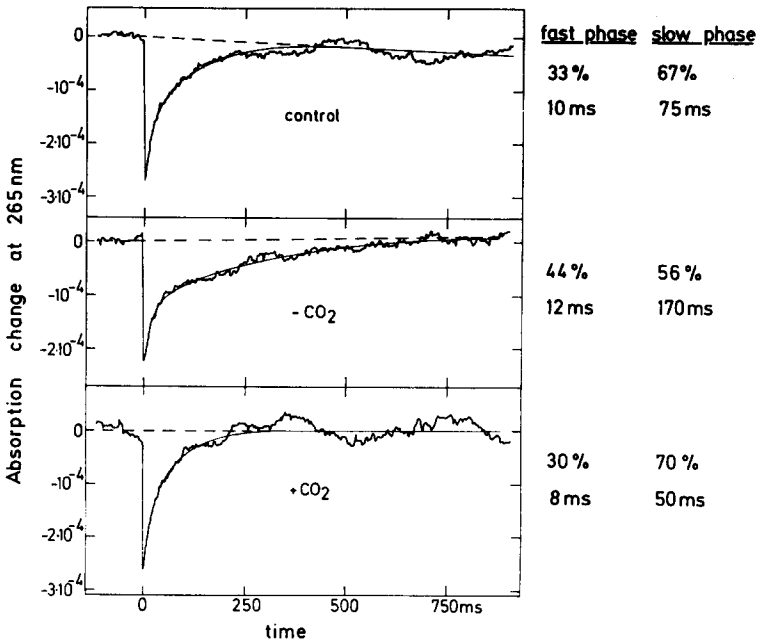


Fig. 3. Time course of the absorption change at 265 nm induced by 20 μs repetitive flashes (indicating mainly R^{2-} or PQ^{2-}) for untreated (control), CO_2-depleted ($-CO_2$) and reconstituted ($+CO_2$) chloroplasts (freshly prepared). The amplitudes $\Delta I/I$ are $2.7 \cdot 10^{-4}$, $2.2 \cdot 10^{-4}$ and $2.6 \cdot 10^{-4}$, respectively. The relative magnitude and halftime of the two exponential phases are also shown. Number of flashes, 512; darktime (t_d) = 1.3 s; electrical bandwidth, 1.7 kHz. Intensity of the monitoring light, 150 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} ($\Delta\lambda$ = 5 nm). Data for second illumination, i.e., from 513 to 1024 flashes are shown for the control (increased halftime because of aging).

not limiting (see, however, refs. 15 and 23).

The control chloroplasts of this study (Fig. 3, top) behave somewhat differently than the "normal" chloroplasts. We have separated the relaxation kinetics into a slow and a fast phase: the fast phase is larger (33 %) and slower (halftime, 10 ms) than the medium phase in normal chloroplasts; and the slow phase is also slower than normal (50 ms halftime in the first and 75 ms in a second illumination period).

The major effect (in 70 % of the signal) of CO_2-depletion is the retardation of the slow phase from 50–75 to about 170–230 ms halftime (Fig. 3). This effect is reversible as addition of 10 or 20 mM bicarbonate restores the halftime from approx. 200 to 50 ms. The signals for depleted and reconstituted chloroplasts in Fig. 3 can be quantitatively understood on the basis of 100 % depletion and approx. 30 % inactivation of centers II (deduced from the amplitude of the slow phase, changing from 1.25 to $1.85 \cdot 10^{-4}$). The absorption change due to Q ,with a halftime of 500 μs, in reconstituted and control chloroplasts has not been resolved here due to the electrical bandwidth chosen. In depleted chloroplasts, we should have observed it because it is slowed down to 7 ms. The corresponding new phase is, however, masked by the presence of a 10-ms phase already in the control. The only indication for its existence is that the 10-ms phase becomes larger (from 30 to 44 %).

The interpretation of the 170–230 ms phase in CO_2-depleted chloroplasts may

be the same or different from that in the control (or reconstituted) case for the 50–70 ms phase. Two possibilities exist: (a) if the relaxation of the signal is governed by the oxidation of plastohydroquinone (PQH_2), then the oxidation of PQH_2 has been slowed down in depleted chloroplasts; (b) if the relaxation is determined by the formation of PQ^{2-} from R^{2-}, accompanied by no or a minor absorption change, and the absorption change is brought about by the consecutive fast oxidation of the plastohydroquinone, then the reduction of PQ pool has been retarded. We favor the second possibility because of our earlier experiments [8] on chlorophyll *a* fluorescence. However, from the present data, we cannot claim that the 200-ms time represents a reaction within the electron transfer chain at all. Therefore, experiments at 265 nm with long flashes and at 703 nm with short flashes were performed.

3. Absorption change at 703 nm induced by 20 μs repetitive flashes

The absorption changes of the plastoquinone system and P700 have been measured simultaneously in the same sample 1) to determine whether or not there is heterogeneity in our preparations and 2) to check that the 170–230 ms time does not correspond to an artificial pathway of the electrons directly to ferricyanide.

Both the *P*-700 and the PQ signals consist of a slow and a fast phase in the CO_2-depleted sample (Fig. 4, top) which suggests some inhomogeneity. If the two phases of the PQ signal would indicate consecutive reactions, then the *P*-700 signal should relax with the larger time constant, whereas the smaller time constant should lead to a lag in

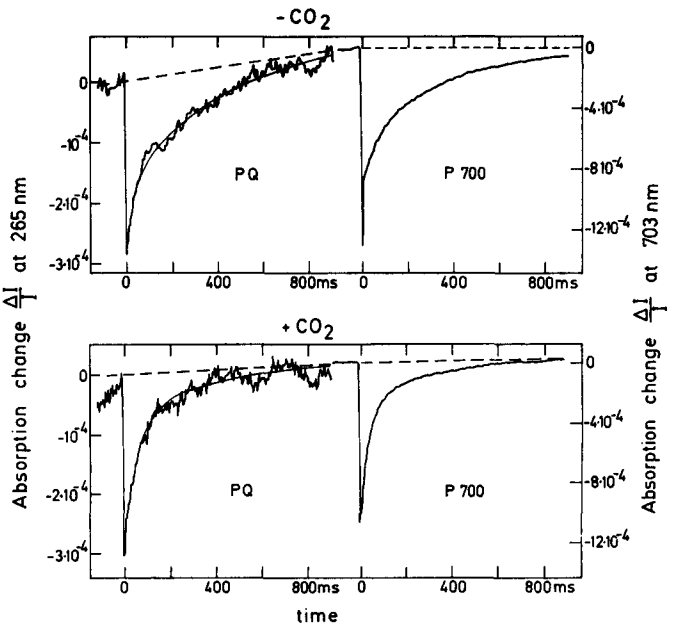


Fig. 4. Absorption changes at 265 (for the PQ system) and 703 nm (for *P*-700) induced by 20 μs repetitive flashes, measured simultaneously, for depleted ($-CO_2$) and reconstituted ($+CO_2$) chloroplasts (frozen and rethawed). The amplitudes are $2.85 \cdot 10^{-4}$ and $3.05 \cdot 10^{-4}$ for the PQ system and $9.1 \cdot 10^{-4}$ and $10 \cdot 10^{-4}$ for *P*-700. Conditions as in the legend of Fig. 3. Intensity of the monitoring light at 703 nm, 70 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} ($\Delta\lambda$ = 5 nm).

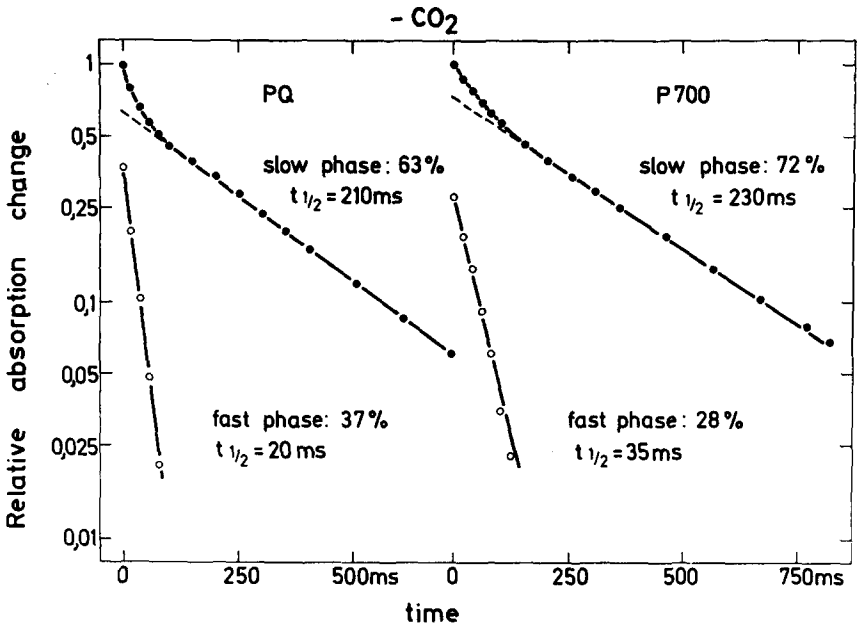


Fig. 5. Semilogarithmic plot of the signals of Fig. 4, top ($-CO_2$ sample). The relative magnitude and halftime of the two exponential phases are indicated for both the PQ system and *P*-700 signal.

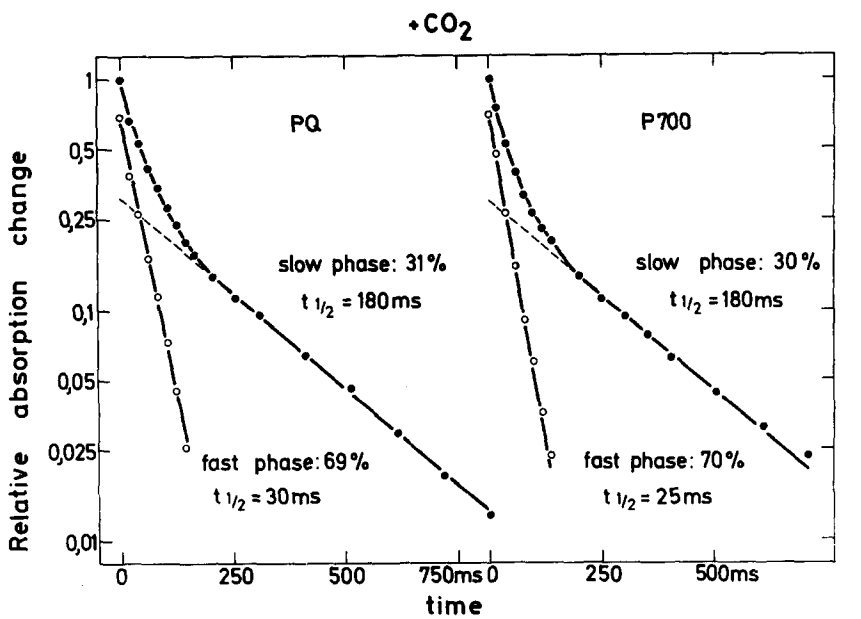


Fig. 6. Semilogarithmic plot of the signals of Fig. 4, bottom ($+CO_2$ sample). The relative magnitude and halftime of the two exponential phases for both the PQ system and *P*-700 are indicated.

the reduction of oxidized *P*-700. This lag is, however, observed only if PQ pool is completely oxidized prior to the flash [17, 20]. The 170–230 ms phase appears with almost equal amplitude (60–70 %) in both the absorption changes of PQ and *P*-700 in the depleted samples (Fig. 5). This means that oxidized *P*-700 is reduced with this halftime. We have not yet shown that this limiting reaction includes the reduction and reoxidation of the plastoquinone pool (the absorption change may be due to R^{2-}). Unfortunately, in these preparations, a small (30 % amplitude) 200-ms phase is also present in the reconstituted samples (Fig. 6) and, probably also in the control. The extent of this phase was increased by the absence of bicarbonate. However, this does not affect our main conclusions.

The quantitative analysis of the signals (Figs. 5 and 6) yields the following picture. The bicarbonate effect is clear: the predominant phase (70 % signal) which has a halftime for both *P*-700 and the plastoquinone system of 210–230 ms in depleted chloroplasts is replaced by a predominant (70 %) phase having a $t_{\frac{1}{2}}$ of 25–30 ms in reconstituted chloroplasts. The fast phase of the *P*-700 signal with a halftime of 25–35 ms represents the transfer of electrons along the normal pathway. The change in the signals induced by CO_2-depletion can be explained by 60 % depletion and 10–20 % inactivation of centers II at 703 and 265 nm respectively. The total amplitude of the 265 nm signal is only slightly reduced by CO_2-depletion because the absorption change due to Q, which was not resolved because of the 500 μs halftime in reconstituted chloroplasts, should now be present to 60 % because of the increased halftime of 7 ms. This new phase has not been separated, but should be included in the fast phase of 20 ms halftime, which therefore is somewhat faster than in the reconstituted case (30 ms) and somewhat larger (37 %) as compared to the *P*-700 signal (28 %).

In conclusion, we have shown in this section that CO_2-depletion slows down the electron transport to *P*-700, from 25–30 ms to 170–230 ms. The participation of the PQ pool molecules and the location of the 200 ms time was best investigated in long flash experiments.

4. *Absorption change at 265 nm induced by 85 ms repetitive flashes*

The qualitative result (Fig. 7) of removal and readdition of bicarbonate is simple: CO_2-depletion reduces the amplitude of the 265 nm signal and slows down its dark relaxation. At first sight, these two effects appear to be contradictory. If the light-induced absorption change would be due to the reduction of PQ pool alone, then the dark relaxation would represent the reoxidation of the plastohydroquinone. Its retardation should be accompanied by an enlarged amplitude, contrary to the experimental result. The observed signals, therefore, cannot be explained simply by the reaction of the PQ-molecules of the pool alone and, therefore, we must include the involvement of R.

If the 265 nm signal is composed of the absorption changes of the transitions PQ/PQ^{2-} and R/R^{2-} two extreme cases may be distinguished:

(1) The elementary reaction of PQ pool-reduction is much faster than the corresponding oxidation. Then, a high degree of reduction in the steady state, i.e., a large absorption change will result. The dark relaxation will be determined by the slow oxidation. The halftime will, however, be altered by the abundance of electrons in the pool and the number of electrons transferred. This case seems to be realized in normal chloroplasts [22] as well as in osmotically shocked chloroplasts [10]. The 25–26 ms

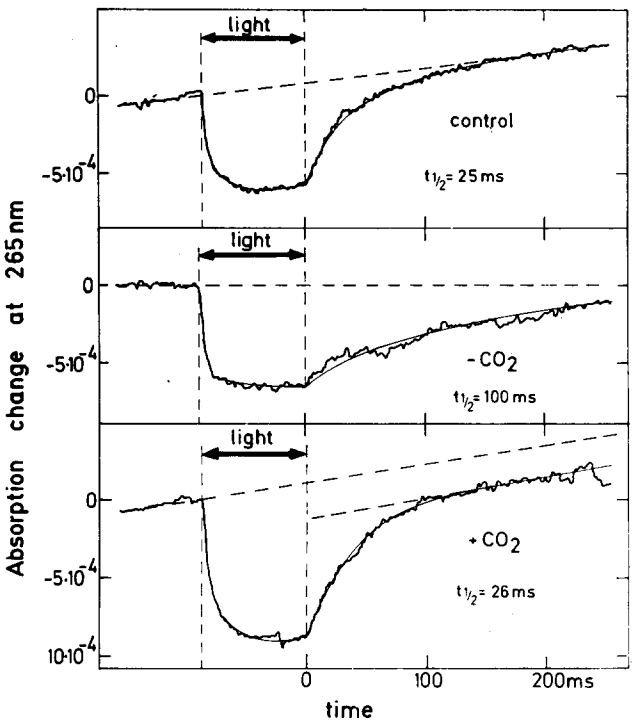


Fig. 7. Time course of the absorption change at 265 nm induced by 85 ms repetitive flashes (indicating mainly R^{2-} and PQ^{2-}) for untreated (control), CO_2 depleted ($-CO_2$) and reconstituted ($+CO_2$) chloroplasts (frozen and rethawed). The amplitudes $\Delta I/I$ are $6.8 \cdot 10^{-4}$, $6.5 \cdot 10^{-4}$ and $9 \cdot 10^{-4}$. respectively. The halftimes of dark relaxation are shown. Number of flashes, 64; darktime (t_d) = 5 s; electrical bandwidth, 600 Hz. 720 nm background light ($\Delta\lambda$ = 15 nm) of 400 ergs · cm^{-2} · s^{-1} intensity was used except in the case of the control (leading to a reduced amplitude in this case).

halftime for dark relaxation here should be due to the oxidation time of PQH_2 (Fig. 7, top and bottom), although it is larger than normal.

(2) The elementary reduction of the PQ pool is slow as compared to its oxidation. Then, a small portion of the pool will be stationarily reduced. The amplitude of the absorption change will be small and mainly due to R^{2-}. During the dark relaxation, R^{2-} will slowly be substituted by PQ^{2-} with subsequent fast oxidation to PQ, so that the process will be governed by the slow reduction of PQ. (The involvement of protons has not been considered in this formulation.) We explain the signals from chloroplasts depleted of bicarbonate (Fig. 7, middle) by this assumption (cf. ref. 8). The interpretation is supported by the fact that the major portion of the signal rises in a rather short time, given by the opening time of the shutter. The Q/Q^--absorption change is not included in the 265 nm long flash signal in case of repetitive excitation. After the first flash PQ^{2-} and R^{2-} will be oxidized, whereas the electron of Q^- will be transferred only to R; R^- will not be oxidized before the next flash [21], so that the absorption change will be only due to the couples R/R^{2-} and PQ/PQ^{2-}.

In the absence of bicarbonate, the reaction constant of the dark relaxation in long flashes should be equal to that of the slow phase in the short flash experiment.

But, the halftime (100 ms) is shorter by a factor of two for unknown reasons. However, the dark relaxation time, suggested to be due to the reduction of PQ, is in fair agreement with the 150 ms suggested earlier from chlorophyll *a* fluorescence measurements [8]. By addition of bicarbonate, the halftime is brought back to 25 ms (Fig. 7 bottom). The amplitude increases by 40 % only. Unfortunately, a comparison with the control signal is not possible. In spite of the high concentration of gramicidin used, the measurement was distorted by scattering changes, perhaps due to the presence of membrane permeating formate. The signal presented in Fig. 7 was obtained with chloroplasts where aging had reduced the scattering changes and omission of far red light had reduced the amplitude, so that only the dark relaxation is apt for comparison. The experimental data indicate that either the capability to accumulate electrons in the PQ-pool is low for the chloroplasts prepared according to the method used here, or that this capability is poorly restored by readdition of bicarbonate. The latter disagrees with the fact that the control dark relaxation of the 265 nm absorption change induced by short and long flashes can be restored by bicarbonate addition.

To exclude possible effects of variable depletion on amplitudes and halftimes, the absorption changes at 265 nm induced by short and long flashes were measured on the same batch of depleted chloroplasts (data not shown). The result does not differ markedly from that discussed above for which different depletions were used. In the depleted chloroplasts, a major phase of 230 ms in the short flash regime corresponds to an 85 ms phase in the long flash regime. This difference requires further investigation, but it does not affect our major conclusions.

CONCLUDING REMARKS

In conclusion, most of the major bicarbonate effects on chloroplast reactions, measured to date, are consistent with the suggestion that both the reduction and oxidation of R are drastically, but reversibly, decreased by bicarbonate depletion. The halftime of reduction of the oxidized PQ-pool is between 100 and 200 ms in the absence of bicarbonate. In normal chloroplasts, the corresponding halftime is not greater than 3 ms, as observed from the lag in the reduction of oxidized *P*-700 after a short flash [17]. This alteration may be due to a structural change of the components to which R is bound or may be a consequence of an unfavorable position of the reactants R and PQ.

Analysis of a large number of experiments showed that the inactivation of reaction center II by bicarbonate depletion varies from 5 to 40 %. Some of the differences between different experiments can be explained to be due to different degrees of depletion. For a biochemical confirmation of the site of bicarbonate action between Q and PQ, see Khanna et al. [24].

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